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# The effect of ‘Astressin’, a novel antagonist of corticotropin releasing hormone (CRH), on CRH-induced seizures in the infant rat: comparison with two other antagonists

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## Abstract

Corticotropin releasing hormone (CRH) has both neuroendocrine effects, promoting ACTH release from the anterior pituitary, and neurotransmitter properties, acting on specific neuronal populations. A recently designed CRH analogue has been shown to be highly potent in preventing activation of pituitary CRH receptors. The efficacy of this compound, Astressin’, in blocking the effects of CRH in the central nervous system (CNS) has not been determined. CRH induces prolonged amygdala-origin seizures in neonatal and infant rats. This model was used in the current study, to compare Astressin to alpha-helical CRH-(9–41), and to [D-Phe<sup>12</sup>, Nle<sup>21,38</sup>, C-MeLeu<sup>37</sup>]CRH-(12–41), ie D-Phe-CRH-(12–41). Astressin (3 or 10  $\mu$ g) was infused into the cerebral ventricles of infant rats prior to CRH infusion. Both doses of the analogue significantly delayed the onset of CRH-induced seizures when given 15, but not 30 min before CRH. No effect of the lower Astressin dose on seizure duration was demonstrated; the higher dose prevented seizures in 2/12 rats, and delayed seizure onset in the others ( $22.7 \pm 5$  min vs  $10.1 \pm 1.3$  min). In the same paradigm, 10  $\mu$ g of alpha-helical CRH-(9–41) and 5  $\mu$ g of D-Phe-CRH-(12–41) had comparable effects on seizure latency and duration. Electroencephalograms confirmed the behavioral effects of Astressin. Therefore, in a CNS model of CRH-mediated neurotransmission, the potency of Astressin is not substantially higher than that of alpha-helical CRH (9–41) and D-Phe-CRH-(12–41).

## Keywords

CRF; receptor blockers; neuropeptide; animal model; EEG

## Introduction

Corticotropin releasing hormone (CRH) is a 41-amino acid neuropeptide which activates target pituitary cells and neurons via specific membrane receptors.<sup>1–3</sup> CRH release during the hormonal response to stress induces the secretion of corticotropin (ACTH) from the pituitary, an action blocked by analogues which compete for the receptors.<sup>4–6</sup> In the central nervous system, CRH mediates ‘central’ components of the stress response, activating neurons in amygdala as well as locus ceruleus, hippocampus and cerebellum.<sup>7–10</sup> In the

immature rat, synthetic CRH produces prolonged limbic seizures.<sup>11–13</sup> EEG mapping has been used to localize the origin of these seizures to the amygdala.<sup>12</sup>

Astressin, an analogue of CRH, {cyclo(30–33)[D-Phe<sup>12</sup>, Nle<sup>21,38</sup>, Glu<sup>30</sup>, Lys<sup>33</sup>]-rat/human-CRH-(12–41), was designed and found to be approximately 30 times more potent than [D-Phe<sup>12</sup>, Nle<sup>21,38</sup>]CRH-(12–41) which is equipotent to [D-Phe<sup>12</sup>, Nle<sup>21,38</sup>, C-MeLeu<sup>37</sup>] CRH-(12–41),<sup>6</sup> on ACTH release, *in vitro*. Astressin was also the most potent CRH analogue for blocking ACTH release in stressed or adrenalectomized rats *in vivo*.<sup>14</sup> The goal of this study was to test the efficacy of Astressin in preventing CRH-induced seizures, a model of the ‘central’ effects of CRH.

## Materials and methods

### Animals

Infant rats ( $n = 111$ ) were offspring of time-pregnant, Sprague–Dawley rats. They were born in our federally-approved animal facility, kept on a 12-h light/dark cycle and given access to unlimited food and water. The time of birth of pups was determined every 12 h, and the day of birth was considered day 0. Litters were culled to 12 pups and mixed among experimental groups. Cages were maintained in a quiet, uncrowded room. Pups were implanted with stainless steel cannulae 24 h prior to experiments,<sup>11</sup> and cannula position was verified in all cases. Peptide infusion was carried out on postnatal days 9–13. Each pup was subjected to CRH or an antagonist once only.<sup>12</sup>

### Materials

CRH, alpha-helical CRH-(9–41) and [D-Phe<sup>12</sup>, Nle<sup>21,38</sup>, C-MeLeu<sup>37</sup>]CRH-(12–41) (ie D-Phe-CRH- (12–41)), were purchased from Bachem (Torrance, CA, USA), Astressin, {cyclo(30–33)[D-Phe<sup>12</sup>, Nle<sup>21,38</sup>, Glu<sup>30</sup>, Lys<sup>33</sup>]-rat/human CRH-(12–41), was synthesized as described elsewhere.<sup>14</sup> Astressin was dissolved in distilled water. To achieve a  $10 \mu\text{g} \mu\text{l}^{-1}$  concentration, the solution was warmed briefly to 60°C.

### Experimental design

Experimental groups ( $n = 5–12$ ) received an antagonist or vehicle 15 or 30 min prior to CRH infusion. Peptides were administered, using a micro-infusion pump, into the lateral cerebral ventricle via the indwelling cannula, while the pups were freely moving in a heated Plexiglas chamber.<sup>11–13</sup> CRH dose (0.15 nmol in  $1 \mu\text{l}$ ) was chosen to result in significant (2–3 h long) seizures with a short latency. Subsequent to the infusion of CRH, seizure latency and duration were monitored for a minimum of 180 min. Animals were scored for behavioral limbic seizures occurring during 5-min epochs.<sup>11,15</sup>

The concordance of limbic automatisms and epileptic discharges induced by CRH has been established previously.<sup>12,13</sup> For each dose of Astressin, a separate group of rats was implanted with bipolar electrodes directed to the amygdala, in addition to cannulae.<sup>12</sup> Electroencephalograms (EEGs) were recorded using a Grass 78E polygraph (Grass Instruments Inc, W Warwick, RI, USA), connected via long, flexible wires to freely moving animals as described elsewhere.<sup>11,12</sup> All infusions were carried out at 8–10 am, to avoid the potential effects of circadian variability in endogenous CRH levels.<sup>16</sup>

Statistical analysis was performed using non-parametric tests (Mann–Whitney unpaired two-tailed comparison, INSTAT software) without assumptions regarding the distribution of values.

## Results

Administration of 3  $\mu\text{g}$  of Astressin 30 min prior to CRH did not have a significant effect on seizure latency: time to seizure onset averaged  $11.4 \pm 3.2$  min in rats given Astressin, and  $7.5 \pm 0.8$  min in the control group. Administration of the same dose of Astressin 15 min prior to CRH resulted in a significant delay of the onset of CRH-induced seizures (Table 1). Seizure latency in the Astressin group averaged  $21.7 \pm 3$  min ( $P < 0.05$  vs controls). Seizure duration did not differ significantly among groups. A higher dose of the antagonist, 10  $\mu\text{g}$ , eliminated CRH seizures entirely in two of twelve rats. In the remainder, latency was increased, as was observed for the lower dose ( $22.7 \pm 5.0$  min).

EEG tracings from amygdala leads of 12-day-old rats subjected to Astressin and CRH are shown in Figure 1. Panel (a) shows 'baseline' bipolar amygdala recording from four rats (Nos 1–4). The tracing in panel (b) was obtained 3–6 min after the administration of 10  $\mu\text{g}$  Astressin to rats No. 1 and No. 2. No significant change in background rhythms was observed. Eight minutes after CRH administration to all four rats (panel c), epileptic discharges were evident in recordings from rats No. 3 and No. 4. By 45 min after CRH infusion, seizures were present in EEG tracings of all four rats (panel d).

In a separate group of rats, the administration of Astressin 30 min after CRH infusion did not abolish the seizures (not shown). Rats receiving Astressin had a higher incidence of wet dog shakes than those receiving CRH alone.

Table 2 compares the effects of Astressin and those of established CRH antagonists given 15 min prior to CRH, on CRH-induced seizures. As is evident in the table, Astressin was not significantly superior to alpha-helical CRH-(9–41), or to D-Phe-CRH-(12–41) in either delaying or attenuating CRH-induced seizures.

## Discussion

In this study, the novel inhibitory analogue of CRH, Astressin, was tested for its ability to prevent seizures induced by CRH administered into the lateral cerebral ventricle of infant rats. The model of behavioral and electroencephalographic seizures induced by CRH in neonatal (first postnatal week) and infant rats (second postnatal week) has been established and characterized.<sup>11–13</sup> CRH results in seizures that originate in the amygdala and spread to other limbic structures.<sup>12</sup> The doses required for seizure induction (picomolar range) are insufficient to activate the pituitary, and do not increase plasma glucocorticoids.<sup>11</sup> Elevation of plasma corticosterone is only evident after the onset of the seizures, which are themselves stressful.<sup>11</sup>

CRH-induced seizures are therefore a paradigm of the central effects of the peptide on its receptor(s). The seizures are blocked by CRH antagonists, but not by, for example, glutamate receptor antagonists.<sup>17</sup> The unique susceptibility of the infant rat to doses of CRH that do not cause seizures in the adult rodent<sup>7</sup> may be a consequence of the developmental profile of CRH receptors. Receptor-binding and autoradiography studies<sup>18,19</sup> have shown that CRH binding capacity in whole brain and in limbic structures of the rat peaks on the second postnatal week. We have recently documented that messenger RNA abundance of the first member of the CRH receptor family, CRF<sub>1</sub>,<sup>20–22</sup> displays a developmental profile of temporal and spatial distribution which is consistent with these earlier studies:<sup>23</sup> receptor-mRNA levels are maximal in the amygdala on postnatal day 9, and in the hippocampus on the sixth postnatal day.<sup>23</sup> CRF<sub>1</sub> is therefore a likely candidate for mediating seizures induced by CRH in the infant rat.

CRH-induced seizures provide a paradigm of ‘central’ actions of the peptide, which is independent of its anxiogenic effect and can be easily quantitated, using both behavioral and EEG parameters.<sup>11,12,15</sup> Astressin has been found, using assays of activation of ‘peripheral’ pituitary receptors,<sup>14</sup> to be markedly more potent than D-Phe-CRH-(12–41) and alpha-helical CRH-(9–41). Using the seizure paradigm, this study failed to demonstrate the enhanced potency of Astressin as compared with the other two antagonists. A similar result has been observed in models of the anxiogenic effects of the peptide in adult rats.<sup>24</sup> The reasons for the discrepancy between the ‘central’ and ‘peripheral’ potencies of Astressin are not clear, but this phenomenon has also been demonstrated for alpha-helical CRH-(9–41).<sup>5</sup>

The mechanism for the discrepancy between the potency of Astressin in ‘peripheral’ versus ‘central’ assay systems is not obvious. The convulsive effects of CRH are likely mediated by the first type of CRH receptors, CRF<sub>1</sub>.<sup>20–22</sup> The binding of Astressin to that receptor ( $K_i = 2.0$  nM) is at least ten-fold higher than that of older analogues, excluding decreased receptor affinity as a cause of the peptide’s relatively low central potency.<sup>14</sup> Interaction with a CRH-binding protein with sequestration of the analogue away from the receptor site should be considered.<sup>25</sup> However, Astressin has very low affinity ( $K_i > 1000$  nM) for the plasma CRH-binding protein.<sup>14</sup> Further, in the current study, the analogue was infused directly into the cerebrospinal fluid of the lateral ventricles. It is possible that Astressin may fail to reach target receptors in the amygdala. Diffusion from the lateral ventricles to target neurons may be hindered by the more rigidly maintained alpha-helical structure of this analogue.<sup>14</sup> Alternatively, binding to other neuronal or glial cell-surface molecules cannot be excluded.

In summary, Astressin, a novel inhibitory analogue of CRH, is exceptionally potent in blocking activation of CRH receptors in the pituitary, but not in eliminating the convulsant effects of CRH on central nervous system receptors.

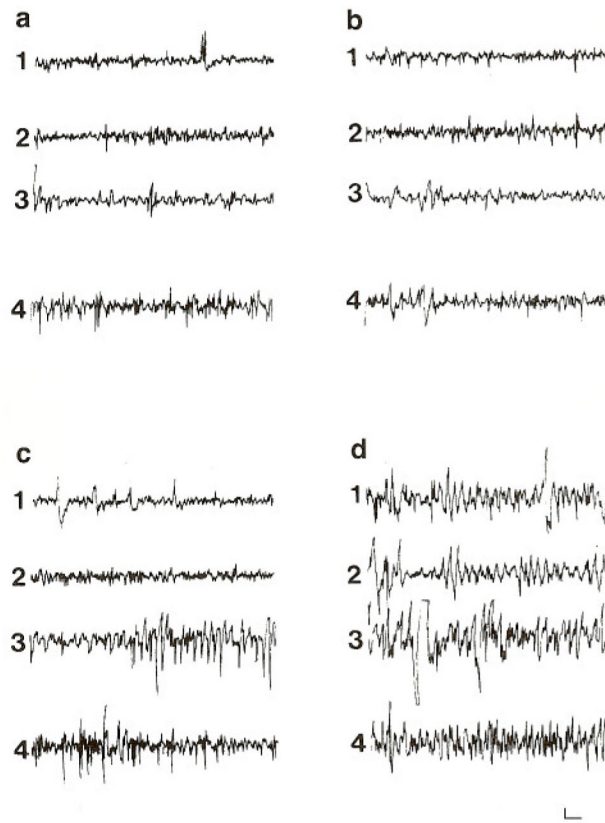
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## References

1. Vale W, Spiess J, Rivier C, Rivier J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and Beta-endorphin. *Science*. 1981; 213:1394–1397. [PubMed: 6267699]
2. Vale W, Rivier C, Brown MR, Spiess J, Koob G, Swanson L, Bilezikjian L, Bloom F, Rivier J. Chemical and biological characterization of corticotropin releasing factor. *Rec Prog Horm Res*. 1983; 39:339–375.
3. De Souza, EB.; Kuhar, MJ. Corticotropin-releasing factor receptors: autoradiographic identification. In: Martin, JB.; Barchas, JD., editors. *Neuropeptides in Neurologic and Psychiatric Disease*. Raven Press; New York: 1986. p. 179-198.
4. Rivier J, Rivier C, Vale W. Synthetic competitive antagonists of corticotropin releasing factor: effect of ACTH secretion in the rat. *Science*. 1984; 224:889–891. [PubMed: 6326264]
5. Fisher L, Rivier C, Rivier J, Brown M. Differential antagonist activity of helical CRF<sub>9–41</sub> in 3 bioassay systems. *Endocrinology*. 1991; 129:1312–1316. [PubMed: 1651845]
6. Hernandez JF, Kornreich W, Rivier C, Miranda A, Yamamoto G, Andrews J, Tache Y, Vale W, Rivier J. Synthesis and relative potencies of new constrained CRF antagonists. *J Med Chem*. 1993; 36:2860–2867. [PubMed: 8411001]
7. Ehlers CL, Henriksen SJ, Wang M. Corticotropin releasing factor produces increases in brain excitability and convulsive seizures in rats. *Brain Res*. 1983; 278:332–336. [PubMed: 6605787]

8. Aldenhoff JB, Gruol DL, Rivier J, et al. Corticotropin-releasing factor decreases postburst hyperpolarization and excites hippocampal neurons. *Science*. 1983; 221:875–877. [PubMed: 6603658]
9. Valentino RJ, Page ME, Curtis AL. Activation of noradrenergic locus ceruleus neurons by hemodynamic stress is due to local release of CRF. *Brain Res*. 1991; 555:25–34. [PubMed: 1933327]
10. Fox EA, Gruol DL. CRF suppresses the after hyperpolarization in cerebellar Purkinje neurons. *Neurosci Lett*. 1993; 149:103–107. [PubMed: 8469370]
11. Baram TZ, Schultz L. CRH is a rapid and potent convulsant in the infant rat. *Dev Brain Res*. 1991; 61:97–101. [PubMed: 1914160]
12. Baram TZ, Hirsch E, Snead OC III, Schultz L. CRH induced seizures in the infant brain originate in the amygdala. *Ann Neurol*. 1992; 31:488–94. [PubMed: 1596084]
13. Baram TZ, Ribak CE. Peptide-induced infant status epilepticus causes neuronal death and synaptic reorganization. *NeuroReport*. 1995; 6:277–280. [PubMed: 7756609]
14. Gulyas J, Rivier C, Perrin M, Koerber SC, Sutton S, Corrigan A, Lahrchi SL, Craig AG, Vale W, Rivier J. Potent structurally constrained agonists and competitive antagonists of CRF. *Proc Natl Acad Sci*. 1995; 92:10575–10579. [PubMed: 7479843]
15. Baram TZ, Schultz L. ACTH does not control neonatal seizures induced by the administration of exogenous corticotropin releasing hormone. *Epilepsia*. 1995; 36:174–178. [PubMed: 7821275]
16. Watts AG, Swanson LW. Diurnal variation in the content of CRH-mRNA in the hypothalamic PVN of rats of both sexes as measured by ISH. *Endocrinology*. 1989; 125:1734–1738. [PubMed: 2788078]
17. Baram TZ, Avishai-Eliner S, Schultz L. Seizure threshold to kainic acid in infant rats is markedly decreased by corticotropin releasing hormone. *Epilepsia*. 1995; 36(supp):abs B-05.
18. Insel TR, Battaglia G, Fairbanks DW, De Souza EB. The ontogeny of brain receptors for corticotropin-releasing factor and the development of their functional association with adenylate cyclase. *J Neurosci*. 1988; 8:4151–4158. [PubMed: 2846796]
19. Pihoker C, Cain ST, Nemeroff CB. Postnatal development of regional binding of corticotropin-releasing factor and adenylate cyclase activity in the rat brain. *Prog Neuro-Psychopharmacol & Biol Psychiat*. 1992; 16:581–586.
20. Wong ML, Licinio J, Gold PW. Localization of CRH receptor mRNA in adult rat by ISH. *Endocrinology*. 1994; 135:2275–2278. [PubMed: 7956950]
21. Potter E, Sutton S, Donaldson C, Chen R, Perrin M, Lewis K, Sawchenko PE, Vale WW. Distribution of CRF-receptor mRNA expression in the rat brain and pituitary. *Proc Natl Acad Sci*. 1994; 91:8777–8781. [PubMed: 8090722]
22. Lovenberg TW, Liaw CW, Grigoriadis DE, Clevenger W, Chalmers DT, De Souza EB, Oltersdorf T. Cloning and characterization of functionally distinct CRF receptor subtype from rat brain. *Proc Natl Acad Sci USA*. 1995; 92:836–840. [PubMed: 7846062]
23. Avishai-Eliner S, Yi SJ, Baram TZ. Developmental profile of CRH-receptor messenger RNA in the rat limbic system. *Dev Brain Res*. 1996; 91:159–163. [PubMed: 8852365]
24. Spina M, Menzaghi F, Risborough V, Britton KT, Merlo-Pich E, Rivier J, Koob GE. The behavioral effect of central administration of the novel CRF antagonist Astressin. *Soc Neurosci Abst*. 1995; 25:304.6.
25. Potter E, Behan D, Linton EA, Lowry PJ, Sawchenko PE, Vale WW. The central distribution of a CRF-binding protein predicts multiple sites and modes of interaction with CRF, *Proc. Natl Acad Sci*. 1992; 89:4192–4196.



**Figure 1.**

Electroencephalogram (EEG) obtained from bipolar leads directed at the amygdala of four 12-day-old infant rats (1–4) Panel (a) shows baseline recording. Panel (b) was recorded 3–6 min subsequent to the infusion of 10  $\mu$ g Astressin to rats No. 1 and No. 2. No significant change in EEG is evident. Eight minutes after the infusion of CRH (0.15 nmol) to all four rats, panel (c) demonstrates epileptiform discharges (which were accompanied by automatisms) in rats No. 3 and No. 4. Rats No. 1 and No. 2 have normal EEGs. Panel (d), recorded 45 min after CRH administration shows epileptiform discharges in all four rats. Horizontal bar represents 1 s; vertical bar represents 50  $\mu$ V.

**Table 1**

Effect of Astressin on parameters of CRH-induced seizures

Antagonist	n	Latency of seizures (min)	Seizure duration (min)
None	11	7.5 ± 0.8	237 ± 18
Astressin-30 min	5	11.4 ± 3.2	193 ± 42
Astressin-15 min	12	21.7 ± 3.0	190 ± 33



**Table 2**

Comparison of the effects of three CRH antagonists on latency and duration of CRH-induced seizures

Antagonist	n	Latency (min)	Duration (min)
None	11	7.1 ± 0.8	108.5 ± 13.5
Alpha-helical CRH-(9–41) (10 µg)	12	No seizures (4/12) 21.4 ± 4.4 (8/12)	52.2 ± 14.4
Antagonist effect		14.3 min delay (67%) No seizures (33%)	56 min reduction ( <i>P</i> = 0.014)
None	9	9.11 ± 1.54	137.8 ± 16.6
D-Phe CRH-(12–41) (5 µg)	11	41.3 ± 11.8 (10/11) No seizures (1/11)	70.8 ± 15.4
Antagonist effect		32.2 min delay (90%) No seizures (10%)	67 min reduction ( <i>P</i> = 0.012)
None	11	7.5 ± 0.9	238.2 ± 20
Astressin (3 µg)	12	21.7 ± 3.1	190.8 ± 34.2
Antagonist effect		13.7 min delay	48 min reduction ( <i>P</i> = 0.34)
None	10	10.1 ± 1.3	146.0 ± 14.8
Astressin (10 µg)	12	22.7 ± 5.0 (10/12) No seizures (2/12)	92.5 ± 20.3
Antagonist effect		12.6 min delay (83.5%) No seizures (16.5%)	53.5 min reduction ( <i>P</i> = 0.037)